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(71) Applicant *(for all designated States except US)*: **MEDICAL RESEARCH COUNCIL** [GB/GB]; 20 Park Crescent, London W1N 4AL (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **VEPRINTSEV, Dmitry, Borisovich** [RU/GB]; 11 Banff Close, Cambridge CB4 2XG (GB).

(74) Agent: **KEITH, W. NASH & CO**; 90-92 Regent Street, Cambridge CB2 1DP (GB).

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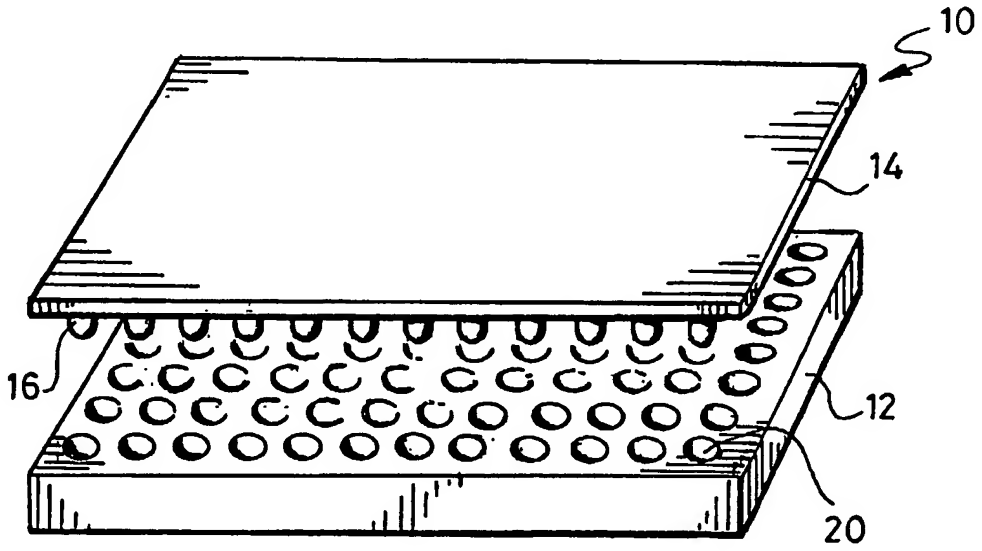
(54) Title: SIMPLE PLATE COVER AND METHOD OF USE THEREFOR



(57) **Abstract:** A sample plate assembly has a sample plate (12) with an array of wells (20) for receiving fluid samples to be analysed. A cover (14) for placing over the plate (12) has, on its underside and in register with the wells (20), an array of protrusions (16) which extend in to the wells (20) so as to make contact with the samples, thereby avoiding an air gap between the ample and cover, thus improving sample analysis.

WO 02/087763 A1

SAMPLE PLATE COVER AND METHOD OF USE THEREFOR

Field of Invention

This invention relates to a cover for a sample plate, such as a 96 well microplate, and a method of using a sample plate with a cover.

Background to the Invention

Sample plates, often known as microplates, are used with associated analysis equipment for analysing a plurality of samples at the same time, each sample being contained within a depression or well in the plate. Whilst typically a microplate contains 96 wells, any number of wells can be provided with the dimensions of the microplate adjusted accordingly, and plates can contain up to 1536 wells.

Advances in electronics and signal processing allow analysis equipment, such as fluorescence and absorbance microplate readers, to be built with a sensitivity comparable with conventional cuvette-based instruments. However use of such analysis equipment is restricted, or not possible, where signals from the samples are of low intensity and variations in signal due to slight differences in the shape of the wells are significant relative to the signal obtained. For example when measuring absorbance and fluorescence of identical samples in the wells, the absorbance and fluorescence characteristics of each sample should be the same. However, slight well-to-well variations affect the characteristics of the emitted radiation, such that identical samples within different wells on a microplate produce different signals.

It is the aim of the present invention to reduce the effect of well-to-well variation for samples contained in a microplate.

Summary of the Invention

In accordance with a first aspect of the present invention, there is provided a cover for a sample plate, such as a microplate, the cover comprising an upper surface and a lower surface, wherein the lower surface carries at least one protrusion extending beyond the lower surface. The protrusion is shaped to extend partway into a well of a sample plate so that in use when a sample is placed within the well and the cover located over the sample plate, the protrusion engages with the sample. This ensures that the sample adjoins the protrusion over much of the diameter of the well substantially eliminating any air gap between the sample and the cover. This also substantially removes any sample meniscus as the air gap between the sample and cover is no longer present. By providing a direct interface between protrusion and sample, well-to-well variation between samples is substantially reduced allowing improved analysis of samples held within the plate.

Preferably a plurality of regularly spaced apart protrusions extend from the lower surface of the cover, the spacing of the protrusions being arranged to be complementary to the spacing of wells within the sample plate. Thus the cover is typically provided with 96 protrusions such that when the cover is placed over a 96-well microplate, each protrusion extends partway into a corresponding well.

The protrusion may in a preferred embodiment be formed as an elongate member, such as a cylinder with a circular cross-section or an elongate cube with a square cross-section. Other forms of the protrusion may also be of advantage.

The elongate member may be formed with a step-wise variation in cross-section, such that an upper part of the member adjoining the lower surface of the cover is of greater diameter than a lower portion of the member engaging with the sample. Where such a step-wise variation is provided in cross-section, the upper part of the protrusion forms a lip which extends beyond a rim of the well and which may seal the sample contents, reducing evaporation of the sample. The seal can be enhanced by placing adhesive or other sealing means, such as a viscous fluid, around the lip.

In another preferred embodiment, the member is formed as an upper cylindrical portion and a lower portion, the lower portion being conical in shape and the upper cylindrical portion extending from the cover.

If required, a region of the upper surface directly above the protrusion may be shaped so as to provide a lens. This allows light to be focussed on the sample, or in reverse to concentrate the emitted signal before reaching analysis equipment.

The cover may be made of plastics material, and in particular injection-moulded plastics material, or quartz, or other suitable transparent material.

In accordance with a second aspect of the invention, there is provided a sample plate assembly comprising a sample plate incorporating at least one depression, or well, for containing a sample, and a cover, wherein the cover has at least one protrusion in correspondence with at least one depression. The protrusion is arranged to fit within the well with a small all-round radial clearance and, in use, to engage with a sample contained in the well, thereby to provide a contiguous sample/cover boundary across a substantial portion of the diameter of the well. This eliminates any gas between the sample and the cover, and thus avoids any distortion effects relating to such gases. Where the sample is a fluid, the meniscus between the sample and the cover is substantially eliminated.

The cover may have the preferred features as discussed above in relation to the first aspect of the invention.

In accordance with a third aspect of the invention, there is provided a method of preparing for analysis samples held in a sample plate, comprising filling or wells within the sample plate with fluid samples to a depth such that on a cover over the wells there is produced a sample/cover interface over a substantial diameter of each well.

The wells may be fully or over-filled, in which case the lower surface of the cover may be planar. Alternatively, the wells may be partially filled and the lower surface of the cover may have protrusions, in accordance with the first aspect of the invention.

The invention will now be described, by way of example, and with reference to the accompanying drawings, in which:

Figure 1 shows a schematic view of a sample plate assembly in accordance with the present invention, the sample plate assembly comprising a sample plate with a plurality of depressions, or wells, and a cover;

Figures 2(a) and 2(b) illustrate a method of over-filling the wells;

Figure 3 is a graph of intensity against wavelength for over-filled wells as shown in Figure 2;

Figure 4 shows a graph of intensity against wavelength for a standard sample plate without use of a cover; and

Figures 5 to 9 show in fragmentary cross-section, five possible shapes of cover and sample plate in accordance with the invention;

Description

Figure 1 shows a microplate assembly 10 comprising a 96-well microplate 12 and a cover 14 with a plurality of protrusions 16, all being made from injection-moulded plastics. The 96-well microplate has 96 wells 20 or depressions regularly spaced in an array of 8 columns by 12 rows. The cover 14 is provided with 96 protrusions 16 in a complementary array. When fluid, solid or viscous samples are placed within the wells 20, the cover 14 is placed over the microplate 12 so as to allow each protrusion 16 to engage with a sample within a respective well. This ensures that the sample is in direct contact with the cover by way of the protrusion and for fluid or viscous samples eliminates any meniscus. The direct interface between sample and cover, with the elimination of any gases between the sample and the cover, reduces the degree of scattering of radiation incident on the sample, or emitted from the sample. Further a uniform top-to-bottom optical path length exists over the diameter of the well for the combined depth of the cover and sample.

With conventional microplates, if identical samples are examined, well-to-well variations result in measurements relating to fluorescence and absorbance varying by approximately 10 per cent. Where the signal emitted by the samples lacks intensity, such variations can prevent meaningful results being obtained.

The well-to-well variation occurs because the uppermost surface of the sample within the well varies in shape, particularly for samples which are fluid in nature and that form a meniscus. The meniscus varies depending on the volume of the sample and hydrophilic/hydrophobic properties of both the sample and the material of the plate. As the wells within the plate are not absolutely identical, the meniscus shape will be slightly different in each well. This then results in a slightly different geometric arrangement for each sample relative to detection and analysis equipment, and thus different levels of signal and scattered light are measured. It is known to use a flat cover in combination with the microplate, but this tends to cause further signal deterioration as this provides yet another surface to scatter light.

The effects of the well-to-well variation can be largely eliminated by ensuring that each sample contacts the cover over most of the diameter of the well.

One way of achieving this is to over-fill each sample well 22 with the fluid samples 24 to be tested, see Figure 2(a), so as to produce a convex meniscus 26 before gently placing a generally planar transparent cover 30 over the plate, as in Figure 2(b). The liquid sample 24 then adjoins the cover 30 as opposed to the prior art where the meniscus is a concave meniscus and the cover and fluid sample do not adjoin but are spaced apart by air.

Each well normally has a volume of 400 µl but in the wells are over-filled with solution to produce a convex meniscus 26 where a small excess of fluid is held by surface tension, see Figure 2(a). Thus each well of a Costar (Costar is a Trade Mark) microplate as sold by Corning, is filled with 0.006@490 nm solution of fluorescein (92 nM) in water using MicroLab M (MicroLab M is a Trade Mark) dispenser as supplied by Hamilton to minimise pipetting error. The cover 30, with a planar lower surface, is then placed gently over the

over-filled wells to eliminate any meniscus, as shown in Figure 2b. Fluorescence measurements are performed on SpectraMAX Gemini XS (SpectraMAX Gemini XS is a Trade Mark) dual-monochromated plate reader, as supplied by Molecular Devices, with an excitation wavelength of 470 nm chosen to separate Rayleigh scattering peak from fluorescein fluourescence and slits fixed at 9 nm. The temperature was 30°C.

The fluorescence spectra 32 for the wells with no air gap between sample and cover i.e. no meniscus, is shown in Figure 3 as a graph of intensity versus wavelength in nm.

By way of comparison, Figure 4 shows fluorescence spectra 34 for wells (24 wells were measured) without covering lid, i.e. from wells from where each sample has a meniscus. Considerable well-to-well variation occurs and it will be seen that for these 24 wells, the variation in intensity is quite considerable.

Thus over-filling the well and placing a cover over the over-filled wells produces a low well-to-well variation with absence of characteristic scatter in the shorter wavelengths, as shown in Figure 3. This is in contrast to Figure 4 where scatter occurs towards the lower wavelengths and well-to-well variation is 11.3 per cent as compared with 1.6 per cent for Figure 3.

Instead of over-filling the wells, the continuous boundary or interface between the samples and the cover can be more reliably achieved by providing a series of integral protrusions 16 extending from the cover, as shown in Figure 1. The protrusions 16 extend partway into each well 20, while still allowing sufficient depth of well to contain an adequate sample for analysis. Various designs of protrusion are shown in Figures 5 to 9. Typically the cover with protrusions is made from injection-moulded plastics material, although it can be made via machining if required.

For the embodiment shown in Figure 5, each protrusion 36 comprises a cylinder extending from the lower surface 40 of the plate. The cylinder is of such a diameter as to fit within the diameter of the well 42 without being a push-fit connection.

The cylindrical protrusion can be shaped at one end to assist in preventing air being trapped between the sample and the protrusion, and thus Figure 6 shows a protrusion 44 comprising two parts, a cylindrical section 46 adjoining the lower surface of the cover and which, at its distal end, tapers to a point so as to provide a second section 50 being generally conical.

In a further embodiment a lens 52 is provided in the upper surface of the cover 14, above each protrusion, as shown in Figure 7. This is achieved by providing a suitably shaped indentation in the upper surface 54 so as to act as a convex lens. This is used to focus light on the sample, or in reverse to concentrate the emitted signal.

Figures 8 and 9 show a cylindrical protrusion 60 having a step-wise variation in diameter, with an upper part 62 of the cylinder having a greater diameter than a lower part 64. This provides a lip 66 extending beyond the diameter of the well, such that when the cover 14 is placed over the microplate a seal is provided reducing evaporation of liquid samples held within the well. Evaporation can be further reduced by placing adhesive 68 or a sealant around the lip 66 so as to provide an air-tight seal to the well, see Figure 9.

The various embodiments described above help reduce evaporation and contamination of samples as the cover can be kept over the plate at all times due to the reduced well-to-well variation. By providing direct contact between the sample and cover, scattering is minimised as any sample/air interfaces and air/cover interfaces are removed. The number of reflective surfaces between the sample and analysis equipment is thus reduced.

CLAIMS

1. A cover for a sample plate, the cover comprising an upper surface and a lower surface, wherein the lower surface carried at least one protrusion extending beyond the lower surface.

2. A cover according to claim 1, wherein the protrusion is shaped to extend partway into a well of the sample plate so that in use when a sample is placed within the well and the cover is located over the sample plate, the protrusion makes contact with the sample.

3. A cover according to claim 1 or 2, wherein a plurality of regularly spaced protrusions extend from the lower surface of the cover, the spacing of the protrusions being arranged to the complementary of the spacing of the wells within the plate.

4. A cover according to claim 3, wherein each protrusion is cylindrical.

5. A cover according to claim 3, wherein each protrusion has a step-wise variation in cross-section, such that an upper part of the protrusion adjoining the lower surface of the cover has a greater diameter than a lower portion of the protrusion.

6. A cover according to claim 5, wherein the step-wise variation defines a lip for engaging an edge of the corresponding well to seal the sample within the well.

7. A cover according to claim 3, wherein each protrusion has an upper cylindrical portion and a lower conical portion.

8. A cover according to any of claims 4 to 7, wherein a region of the upper surface directly above each protrusion is shaped so as to provide a lens.

9. A sample plate assembly comprising a sample plate incorporating at least one well for containing a sample, and a cover having at least one protrusion for registering with the well.

10. An assembly according to claim 9, wherein the cover is in accordance with any of claims 1 to 8.

11. A method of preparing for analysis samples held in a sample plate, the method comprising filling wells in the sample plate with fluid samples to a depth such that on placing a cover over the wells there is produced a sample/cover interface over a substantial proportion of the area of each well.

12. A method according to claim 11, wherein the wells are fully filled or over-filled and the lower surface of the cover is planar.

13. A method according to claim 11, wherein the wells are partially filled and the lower surface of the cover has protrusions which register with the wells and extend into the wells to make contact with the samples.

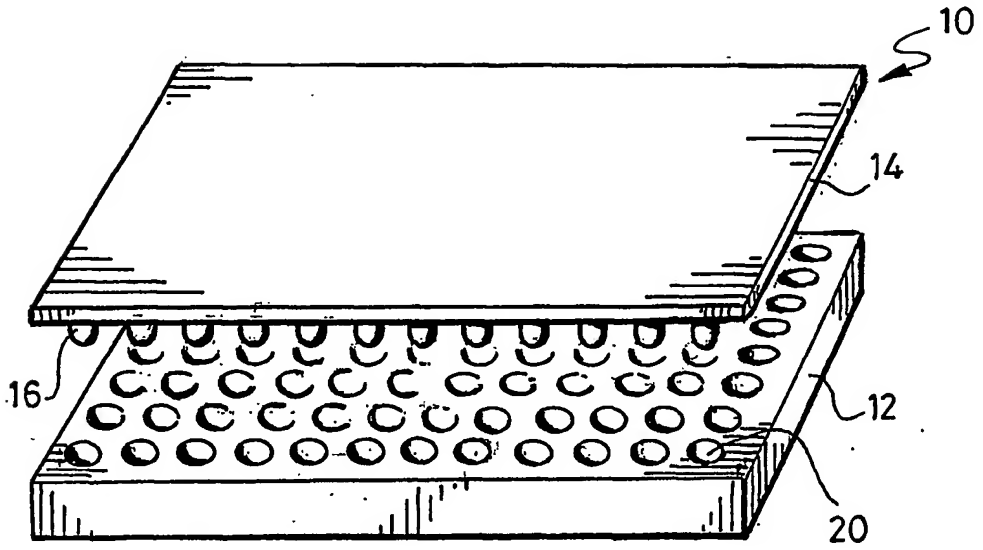


Fig. 1

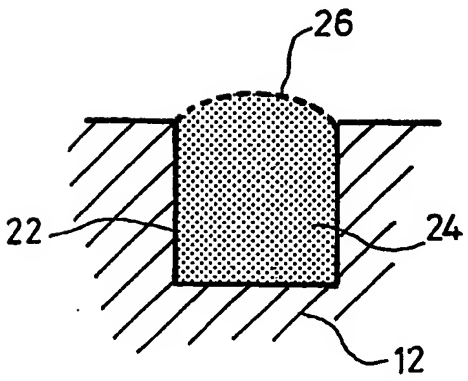


Fig. 2(a)

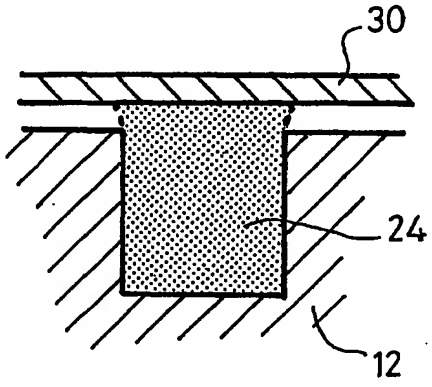


Fig. 2(b)

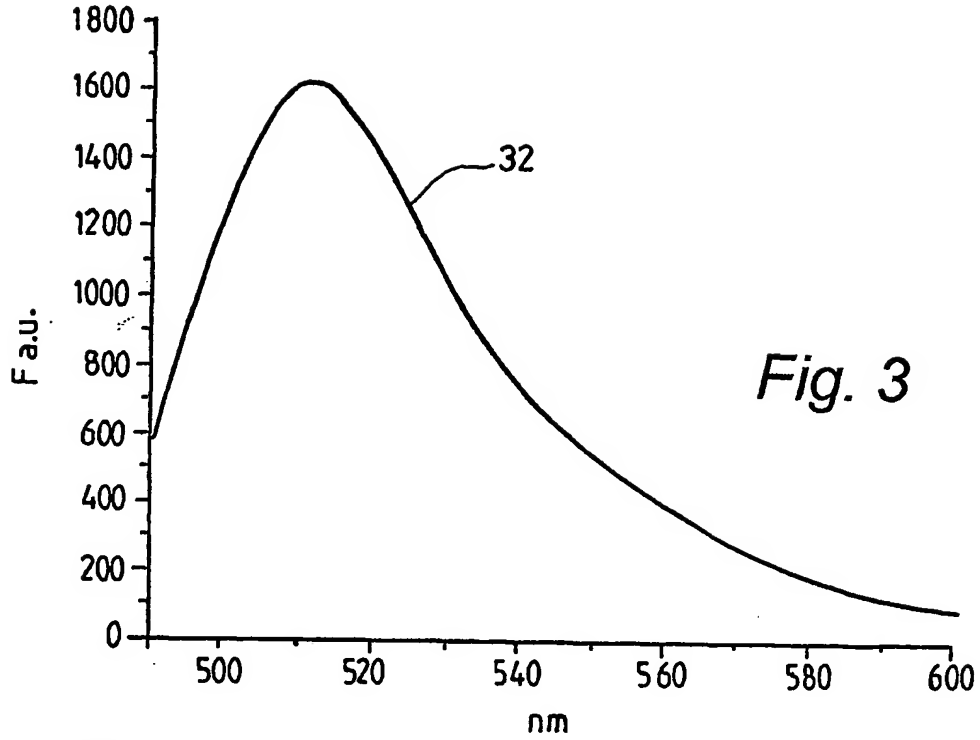

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Fig. 3

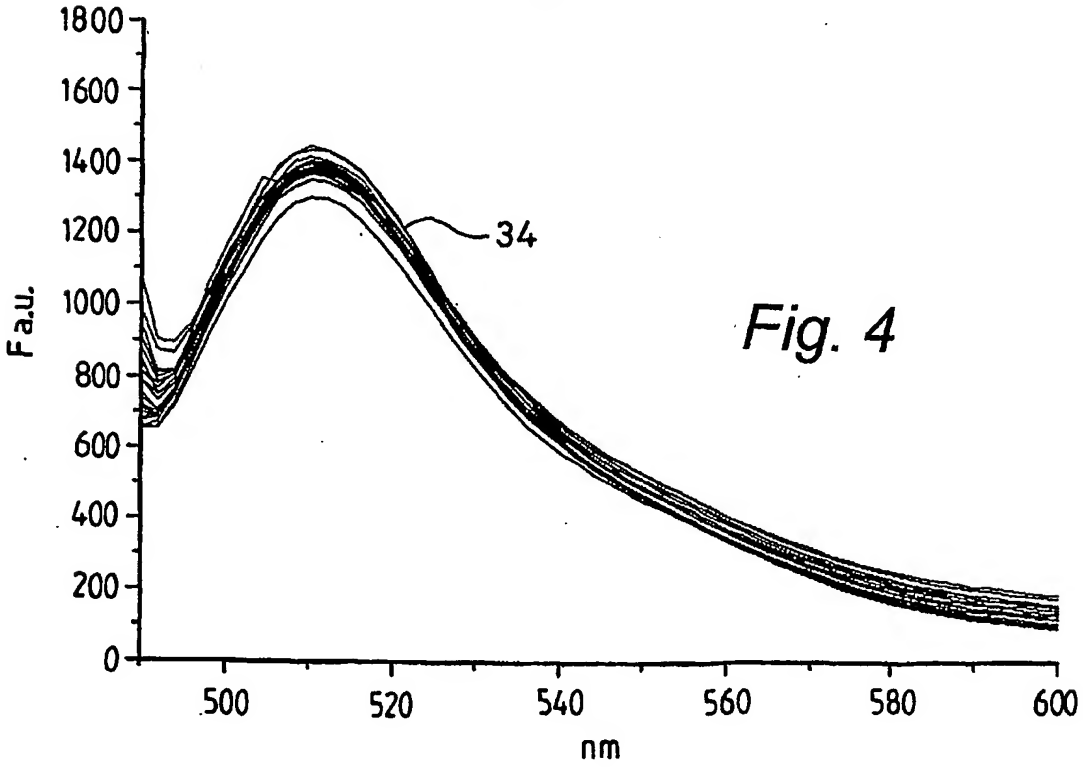
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Fig. 4

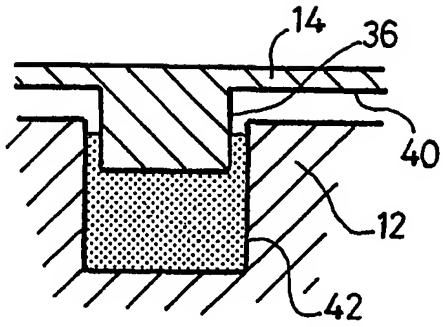

Fig. 5

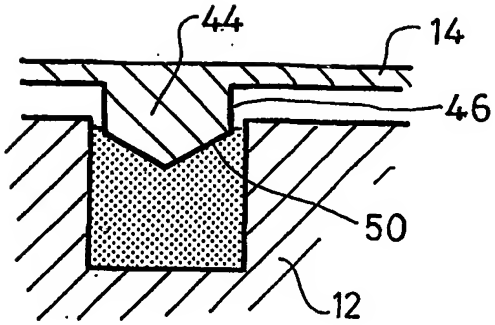

Fig. 6

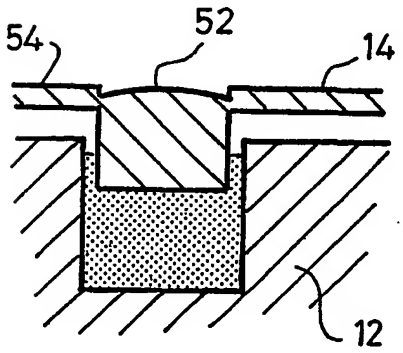

Fig. 7

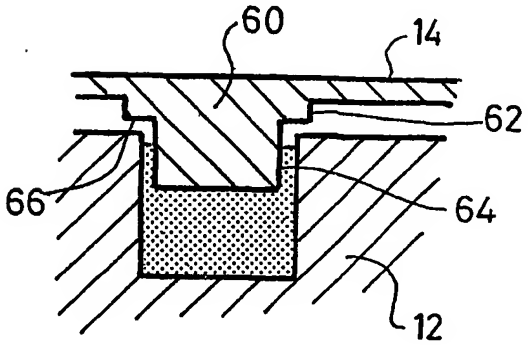

Fig. 8

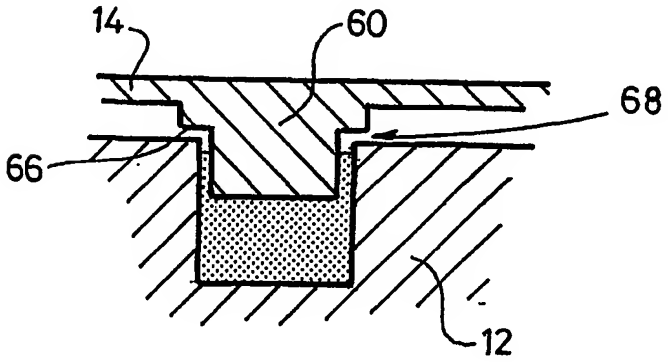

Fig. 9

INTERNATIONAL SEARCH REPORT

PCT/GB 02/01850

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00 G02B21/34 G01N21/03

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G02B B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
13 August 2002	21/08/2002

Name and mailing address of the ISA	Authorized officer
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